Microbiology of Human Spacecraft Environments

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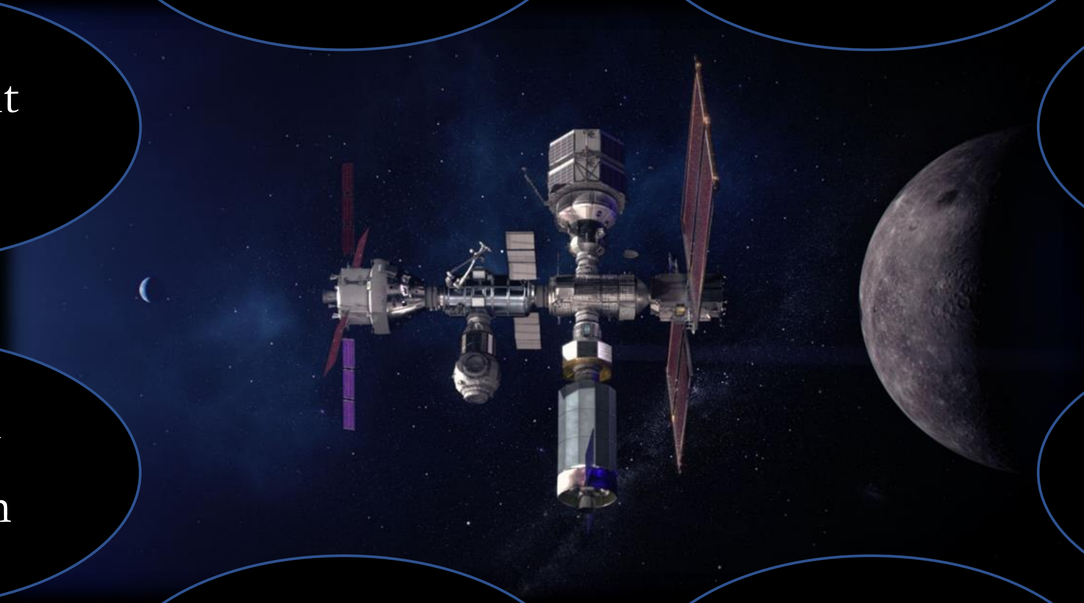
To HAB
PEPPERONI

Commercial Spaceflight

- Commercial Cargo
  - SpaceX
  - Northrup Grumman
  - Sierra Nevada
- Commercial Crew
  - SpaceX
  - Boeing
- Axiom Segment/Axiom Space Station
- Commercial Low Earth Orbit Destinations (CLD) Program
  - Starlab Space Station
  - Orbital Reef

Microbiology and Space Missions

- Astronaut health
- Spacecraft integrity
- Spaceflight foods
- Life support systems



- Planetary protection
- Trash containment
- Hygiene activities
- Human waste containment

Astronaut Health

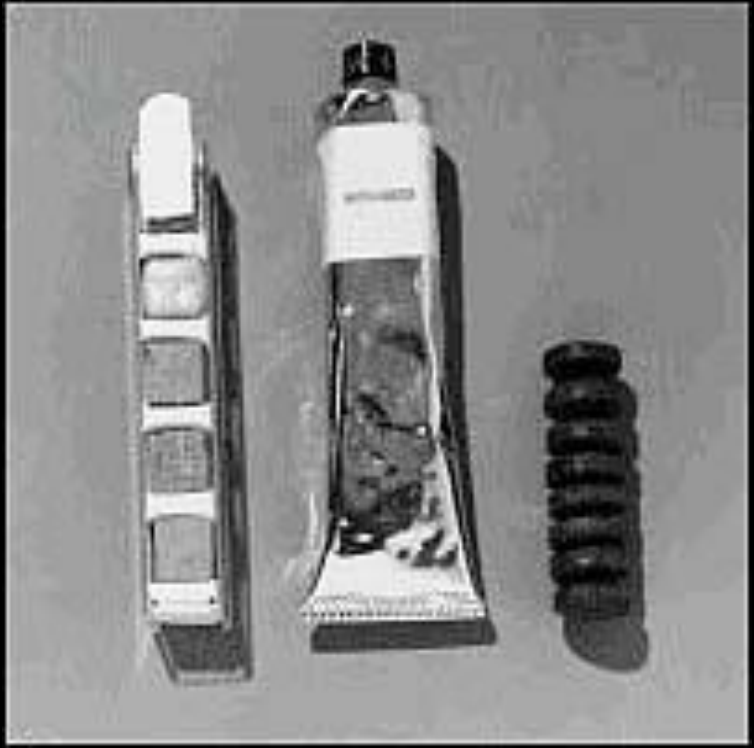
- Diagnosis is often based on symptomology (e.g., headache, rash, dry hacking cough, diarrhea)
- Examples of infectious diseases during spaceflight missions include upper respiratory infections, urinary tract infections, ear infections, herpes zoster, rashes and skin disorders, and gastroenteritis.
- Survey of Space Shuttle missions (STS-1 through STS-89) indicated infectious disease accounted for 1.4% of all medical events (not including skin and subcutaneous tissue)
- Evidence of immune system dysfunction

Why do exceptionally healthy astronauts in a pristine environment still contract infectious diseases?

Risin, D. in Human Health and Performance Risks of Space Exploration Missions, (NASA SP-2009-3405, 2009).

Spaceflight Food

Mercury Program



ISS Program

Deep Space Exploration?

Spaceflight Food

- Several categories
  - Fresh foods
  - Irradiated foods
  - Thermostabilized foods
  - Non-thermostabilized foods
- Non-thermostabilized foods
  - Includes dehydrated foods, tea, food bars, and cheese
  - Samples from each production lot are tested
  - Lots have been disqualified after detection of microbes, including the bacteria *Salmonella enterica* Typhimurium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and fungi *Aspergillus fumigatus*, and *Aspergillus flavus*.

The Future of Spaceflight Food

- Providing nutrient rich, tasty, and safe food that is stable for long-duration exploration missions is challenging.
- Several new concepts are being investigated on the International Space Station (ISS) to enable and enhance future spaceflight missions.
  - "Pick and eat crops", such as lettuce and peppers, are being grown and consumed by the ISS astronauts.
  - The concept of preparing and cooking food on the ISS is being evaluated for future missions.
- New "Categories" of foods are being provided, especially through commercial space providers.

How do we continue to keep space travelers safe as these new food concepts are implemented?

Design of Spacecraft Habitats

- Prevention is the best approach to minimize microbiological problems during spaceflight missions.
- When designing a new spacecraft/habitat, our experience with ISS indicates the risk of microbial contamination can be reduced using:
    - Habitat surfaces that are not conducive to microbial growth
    - High Efficiency Particulate Air (HEPA) filters to maintain good air quality
    - Temperature and humidity controls that minimize condensate formation
    - Preflight microbiological monitoring and disinfection of the vehicle and its cargo
- Microbial control of potable water depends on the source and processing of the water (*e.g.*, recycled humidity and/or urine may require more treatment)

On ISS, recycled water experiences a catalytic oxidizer (267°F for 10 minutes), iodine disinfection, and passes through a 0.2-micron filtration

Operations of Spacecraft Habitats

- Health Stabilization Program
  - Preflight quarantine to decrease the risk of infectious disease
  - Employs other prevention techniques, such as raising infectious disease awareness
- Preflight disinfection and microbial monitoring
- Inflight cleaning and remediation
  - Regular housekeeping
  - Disinfectant wipes for contaminated surfaces
  - High concentration iodine solution to disinfect contaminated potable water systems

Contamination Potential

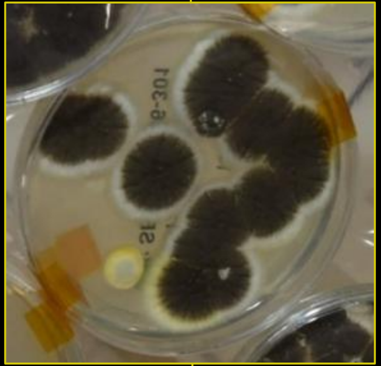
Spacecraft are complex

Trash and waste control

Astronaut activities, such as eating and hygiene

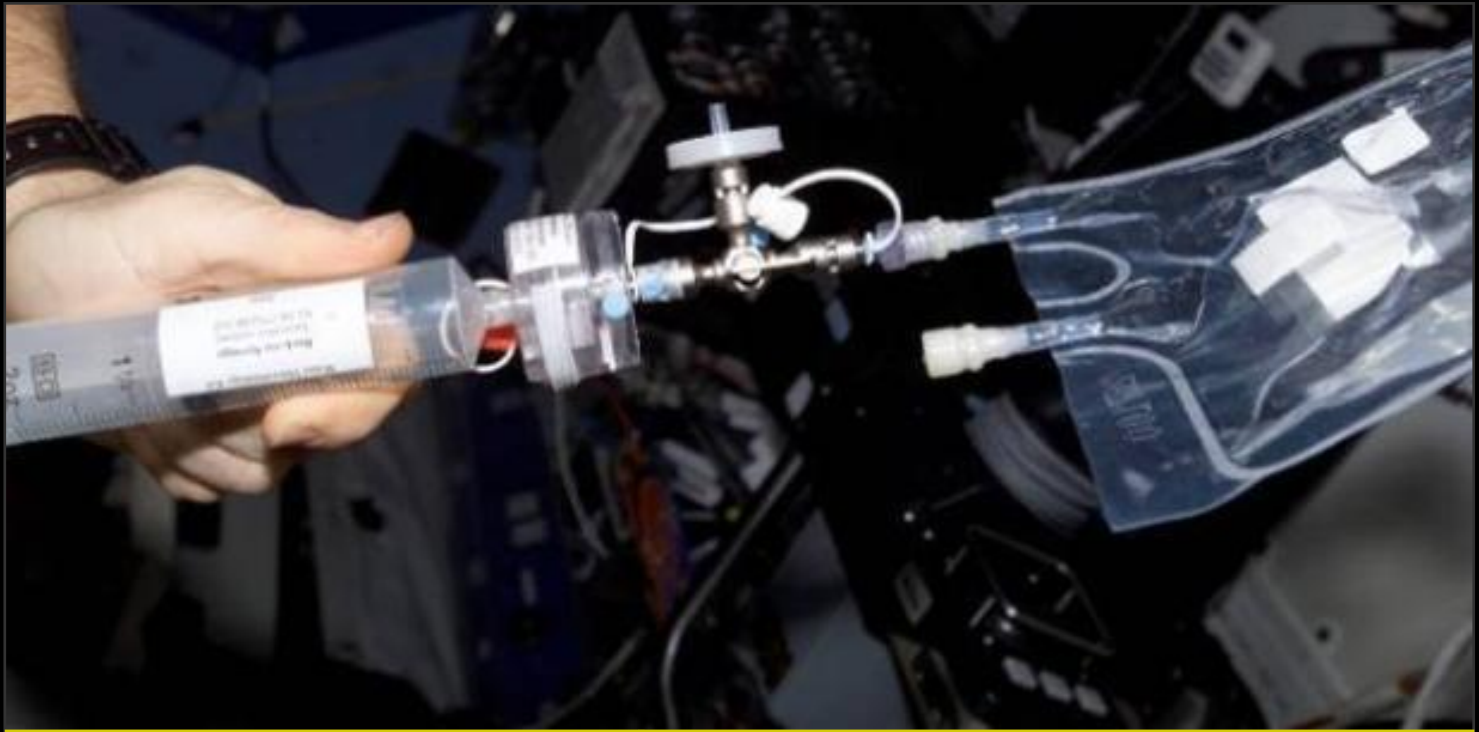
Microbial Monitoring on the ISS

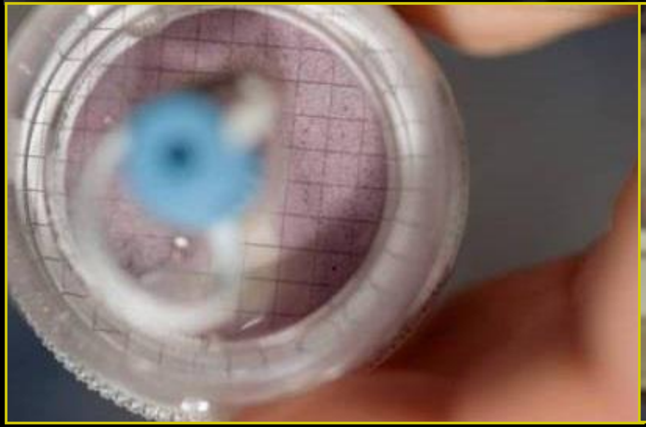
Surfaces | **Air** | **Water**



Quantified in-flight and returned to Earth for identification

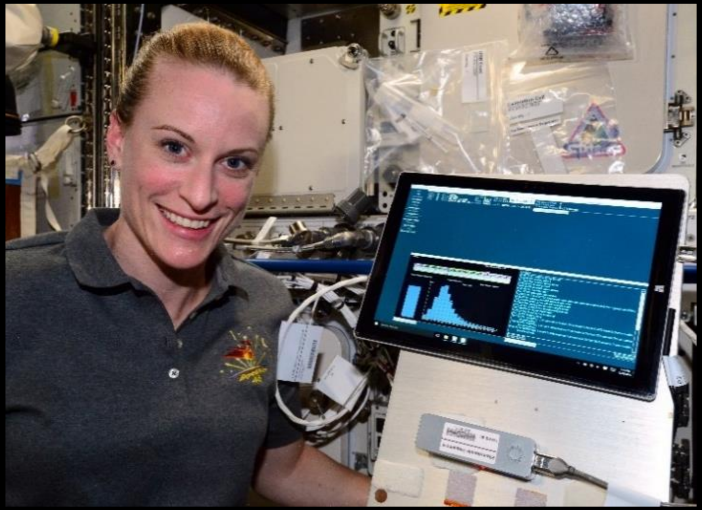
Microbial Monitoring of Water





Advances in Microbial Monitoring

- DNA sequencing in space
    - First performed in 2016 using nanopore sequencing technology
- In-flight identification of environmental samples
    - Three microbial colonies from a media plate used for ISS surface sampling were processed and sequenced on ISS.
    - The sequencer accurately identified the three isolates that were selected.
- Following up with "Swab to Sequencer" direct analysis



S. Stahl-Rommel, *et al.* *Genes* **12**, 106 (2021).

Environmental Health

Microbial monitoring by NASA and others indicate ISS environmental flora reflect human-associated microorganisms commonly found in terrestrial homes.

- Microbiological monitoring occasionally identifies opportunistic bacterial pathogens, such as ***Staphylococcus aureus*** and ***Bacillus cereus***. No methicillin resistant *S. aureus* (MRSA) has been identified.
- ***Klebsiella, Enterobacter*** and ***Enterococcus*** species are occasionally identified throughout the ISS.

D. Pierson, *et al.* in *Environmental Monitoring: A Comprehensive Handbook* (DHI Publishing, 2012)
R. A. Blaustein, *et al. mSystems* **4**, doi:10.1128/mSystems.00281-18 (2019)

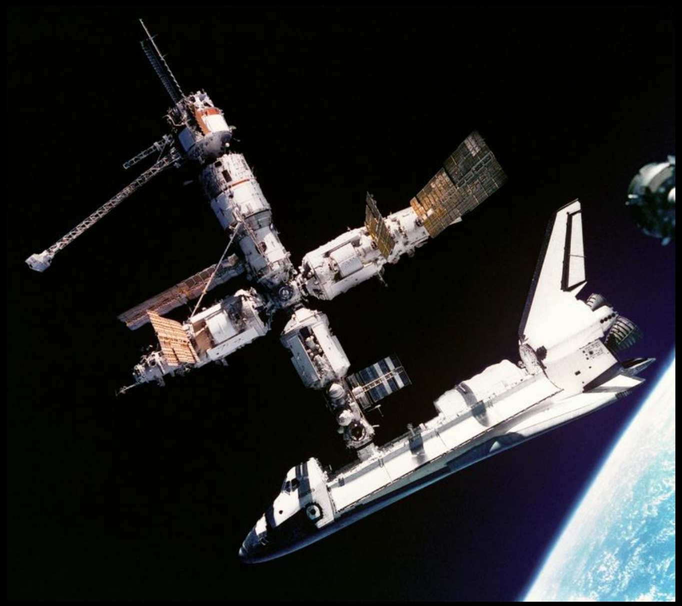
Environmental Health

- The most prevalent fungal genera are ***Aspergillus*** and ***Penicillium***. ***A. flavus***, ***A. niger***, and ***A. fumigatus*** have been identified from spaceflight samples. ***Stachybotrys chartarum*** has been isolated preflight.
- Samples from potable water (for drinking and hygiene) indicate common water-borne bacterial species, including several species from the genera ***Ralstonia***, ***Burkholderia***, and ***Cupriavidus***.

As ISS has many visiting vehicles with astronauts, "new" bacterial and fungal isolates are regularly identified.

Environmental Anomalies

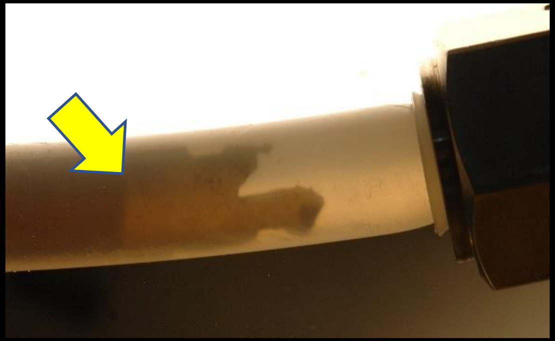
- Russian Mir Space Station (1986-2001)
  - Samples of free-floating condensate caused by power failures and problems with temperature control revealed high microbial diversity, including *Escherichia coli*, *Serratia marcescens*, *Legionella* species, and protozoa
- International Space Station (1998-present)
  - Surface contamination is often associated with uncontrolled water
  - Air contamination and unpleasant odors do occur; however, the sources are often unknown. These events have been transient and/or handled by the HEPA filtration system.
  - Biofilm formation in ISS water lines have caused clogged lines and pump failure.



C. M. Ott, et al. *Microb Ecol* 47, 133-136 (2004)

ISS Environmental Anomalies

Fungal Contamination in ISS Module



Bacterial Biofilm in ISS Drinking Water System

Fungal Plant Contamination

Fungal Contamination in ISS Cabin Air

???

Spaceflight Microbiological Research

- Multiple spaceflight experiments over the past six decades have demonstrated unexpected microbial responses when microorganisms are cultured during spaceflight, including alterations in:
  - Antibiotic resistance
  - Biofilm formation
  - Growth rates
  - Virulence
  - Gene expression
- Rotating Wall Vessel (RWV) bioreactor
  - Fluid movement in the reactor simulates several aspects of the microgravity environment
  - Provides predictive and follow-up studies for spaceflight experiments

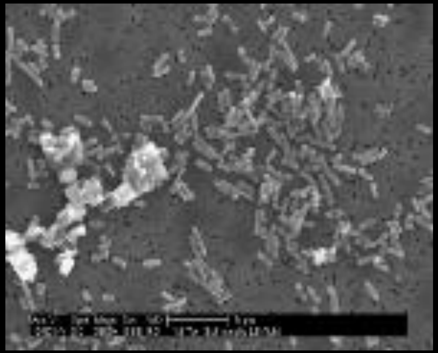
Fluid Processing Apparatus (FPA), an example of in-flight hardware

Low fluid shear culture conditions in the RWV prompted the term Low Shear Modeled Microgravity (LSMMG) environment

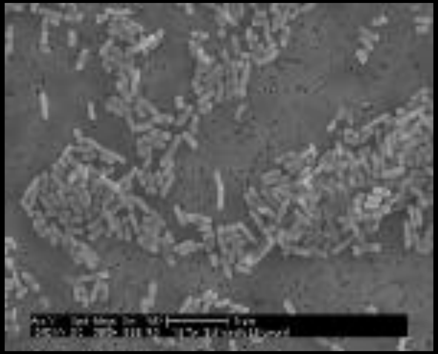
MICROBE: *Salmonella* Response to Spaceflight Culture

PI: Dr. Cheryl Nickerson, Arizona State University

Flight Sample



Ground Control



- In 2006, the MICROBE experiment compared the characteristics of *Salmonella* Typhimurium grown in space to otherwise identical cultures grown on the Earth.
  - First to identify alterations in microbial virulence in a spaceflight grown microorganism
  - First transcriptomic study of a microorganism grown in space
  - First identification of a spaceflight molecular response mechanism
  - First to identify novel changes in biofilm production

Wilson, J. W. *et al. Proc Natl Acad Sci U S A* **104** (2007)

Selected Recent Studies

- PI: Dr. Mark Ott, NASA
  - Co-PI: Dr. Cheryl Nickerson, Arizona State University
  - Determine if growth in the RWV spaceflight analogue (RWV) environment increases virulence in other bacteria, five pathogenic species are being investigated to characterize changes in stress response, adhesion/invasion of 3-D tissue culture models, and animal testing if warranted.

- PI: Dr. Bob McLean, Texas State University
  - Co-PI: Dr. Cheryl Nickerson, Arizona State University
  - Investigate polymicrobial biofilm development by *Pseudomonas aeruginosa* and *Escherichia coli* during spaceflight to identify changes in biofilm architecture, disinfection, and corrosion potential during spaceflight.

- PI: Dr. Cheryl Nickerson, Arizona State University
  - Evaluate the effect of spaceflight on the host-pathogen interaction between human intestinal epithelial cells and *Salmonella* Typhimurium

J. Barrila, *et al. npj Microgravity* (2021)

Selected Recent Studies

- PI: Dr. Cheryl Nickerson, Arizona State University
  - Investigate the combined effect of spaceflight analogue (RWV) culture with lunar dust simulant to determine if the combination synergistically increases the potential infection risk.
- PI: Dr. Hernan Lorenzi, J Craig Venter Institute
  - First comprehensive study of the microbiomes of nine astronauts on ISS, including monitoring of skin sites, nostrils, fecal samples collected preflight, in-flight, and post-flight
  - Identified changes in the astronaut intestinal and skin microbiomes
- PI: Dr. Cheryl Nickerson, Arizona State University
  - Investigate the combined effect of spaceflight analogue (RWV) culture with lunar dust simulant to determine if the combination synergistically increases the potential infection risk.

A. A. Voorhies, *et al. Sci Rep* **9** (2019)

Questions?

NASA Solicitations:
https://nspires.nasaprs.com/external/